

## CLAIMS:

1. A method for determining a concentration of a target nucleic acid by using a nucleic acid probe labeled with a fluorescent dye, which comprises:

5 providing, as said probe, a nucleic acid probe capable of reducing fluorescence emission from said fluorescent dye when hybridized with said target nucleic acid;

hybridizing said probe to said target nucleic acid; and  
measuring a decrease in fluorescence emission from said  
10 fluorescent dye after said hybridization relative to  
fluorescence emission from said fluorescent dye before said  
hybridization.

15 *Sub* 2. A nucleic acid probe for determining a concentration of a target nucleic acid, said probe being labeled with a fluorescent dye, wherein:

said probe is labeled at an end portion thereof with said fluorescent dye, and

20 said probe has a base sequence designed such that, when said probe is hybridized with said target nucleic acid, at least one G (guanine) base exists in a base sequence of said target nucleic acid at a position 1 to 3 bases apart from an end base portion where said probe and said target nucleic acid are hybridized with each other;

25 whereby said fluorescent dye is reduced in fluorescence emission when said probe is hybridized with said target nucleic

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3. A nucleic acid probe according to claim 2, wherein said probe is labeled at a 3' end thereof with said fluorescent dye.

4. A nucleic acid probe according to claim 2, wherein said probe is labeled at a 5' end thereof with said fluorescent dye.

5. A nucleic acid probe for determining a concentration of a target nucleic acid, said probe being labeled with a fluorescent dye, wherein:

said probe is labeled at an end portion thereof with said fluorescent dye, and

said probe has a base sequence designed such that, when said probe is hybridized with said target nucleic acid, base pairs in a probe-nucleic acid hybrid complex form at least one G (guanine) and C (cytosine) pair at said end portion;

whereby said fluorescent dye is reduced in fluorescence emission when said probe is hybridized with said target nucleic acid.

6. A nucleic acid probe according to claim 5, wherein said probe has G or C as a 3' end base and is labeled at said 3' end thereof with said fluorescent dye.

7. A nucleic acid probe according to claim 5, wherein said probe has G or C as a 5' end base and is labeled at said 5' end thereof with said fluorescent dye.

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8. A nucleic acid probe according to claim 4 or 7, wherein a hydroxyl group on a 2' or 3' carbon of a ribose or a 3' carbon of a deoxyribose at a 3' end of said probe has been phosphorylated.

9. A nucleic acid probe according to any one of claims 1-8, wherein an oligoribonucleotide of said probe is a chemically-modified nucleic acid.

10 10. A nucleic acid probe according to any one of claims 2-8, wherein an oligonucleotide of said probe is a chemiric oligonucleotide comprising a ribonucleotide and a deoxyribonucleotide.

11. A nucleic acid probe according to claim 10, wherein said ribonucleotide is a 2'-O-methyloligoribonucleotide.

15 12. A method for determining a concentration of a target nucleic acid, which comprises hybridizing a nucleic acid probe according to any one of claims 2-8 to said target nucleic acid and measuring a decrease in fluorescence emission from said fluorescent dye after said hybridization relative to fluorescence emission from said fluorescent dye before said hybridization.

20 13. A method for determining a concentration of a target nucleic acid, which comprises:

hybridizing a nucleic acid probe according to any one of claims 9-11 to said target nucleic acid, and

25 measuring a decrease in fluorescence emission from said fluorescent dye after said hybridization relative to

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fluorescence emission from said fluorescent dye before said hybridization.

14. A method for analyzing or determining polymorphism or mutation of a target nucleic acid or gene, which comprises:

5        hybridizing a nucleic acid probe according to any one of claims 2-11 to said target nucleic acid or gene, and measuring a change in fluorescence.

10        15. A kit for analyzing or determining polymorphism or mutation of a target nucleic acid or gene, comprising a nucleic acid probe according to any one of claims 2-11.

16. A method for analyzing data obtained by an analysis or determination method according to claim 14, which comprises the following step:

15        correcting a fluorescence intensity of a reaction system, in which said target nucleic acid or gene has been hybridized with said nucleic acid probe labeled with said fluorescent dye, in accordance with a fluorescence intensity of said reaction system before said hybridization.

20        17. A system for analyzing or determining polymorphism or mutation of a target nucleic acid or gene, comprising means for practicing a data analysis or determination method according to claim 16.

25        18. A computer-readable, recording medium comprising a program recorded therein for making a computer perform a correction step as defined in claim 16.

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19. A method according to claim 13, further comprising adding, before said hybridization, a helper probe to a reaction system in which said hybridization is conducted.

5 20. A method according to claim 13, wherein said nucleic acid probe and said target nucleic acid are hybridized after said target nucleic acid is subjected to heat treatment under conditions suited for sufficient degradation of a higher-order structure of said target nucleic acid.

21. A kit according to claim 15, further comprising a helper probe for being added to a hybridization reaction system.

22. A method according to claim 12, 13, 19 or 20, wherein said target nucleic acid is RNA.

23. A device for determining concentrations of nucleic acids, comprising:

a solid support, and

a nucleic acid probe according to any one of claims 2-11 or a different nucleic acid probe bound on a surface of said solid support, said different nucleic acid probe having a structure designed such that said probe comprises two  
20 fluorescent dyes of different kinds in a molecule and that, owing to interaction between said two fluorescent dyes, said probe quenches or emits fluorescence when said probe is not hybridized with said target nucleic acid but emits fluorescence or quenches when said probe is hybridized with said target  
25 nucleic acid;

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whereby said device can determine said concentration of said target nucleic acid by hybridizing said target nucleic acid to said probe or said different probe.

24. A device according to claim 23, wherein said probes or said different probes are arranged and bound in an array pattern on said surface of said solid support.

10 25. A device according to claim 23 or 24, wherein said probes or different probes bound on said surface of said solid support are each independently provided with at least one temperature sensor and at least one heater arranged on an opposite surface of said solid support such that an area of said solid support, where said probe or different probe is bound, can be controlled to meet optimal temperature conditions.

15 26. A device according to any one of claims 23 to 25, wherein said probes or different probes are bound at end portions, where said probes or different probes are labeled with no fluorescent dye, on said surface of said solid support.

20 27. A method for determining a concentration of a target nucleic acid, which comprises determining said concentration of said target nucleic acid by using a device according to any one of claims 23-26.

25 28. A method according to any one of claims 1, 12, 13, 19-22 and 27, wherein said target nucleic acid is a nucleic acid derived from a microorganism or animal and obtained by pure purification.

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29. A method according to any one of claims 1, 12, 13, 19-22 and 27, wherein said target nucleic acid is a nucleic acid contained in cells of a co-cultivation system of microorganisms or symbiotic cultivation system of microorganisms or in a homogenate of said cells.

30. A method for determining a concentration of a nucleic acid amplified in PCR, which comprises:

conducting reactions in PCR by using a nucleic acid probe according to any one of claims 2-3, 5, 6, and 8-11;

measuring an intensity of fluorescence of a reaction system in which at a time of a nucleic acid extending reaction, said probe has been degraded out by polymerase or said reaction system in which a nucleic acid denaturing reaction is proceeding or has been completed and also an intensity of fluorescence of said reaction system in which said target nucleic acid or amplified target nucleic acid has hybridized with said nucleic acid probe; and then

calculating a decrease of said latter intensity of fluorescence from said former intensity of fluorescence.

31. A method for determining a concentration of a nucleic acid amplified in PCR, which comprises:

conducting reactions in PCR by using, as a primer, a nucleic acid probe according to claim 4 or 7;

measuring an intensity of fluorescence of a reaction system in which said probe and said target nucleic acid or

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amplified nucleic acid have not hybridized with each other and also an intensity of fluorescence of said reaction system in which said probe and said target nucleic acid or amplified nucleic acid have hybridized with each other; and then

5 calculating a decrease of said former intensity of  
fluorescence from said latter intensity of fluorescence.

32. A method according to claim 30 or 31, wherein said PCR is real-time quantitative PCR.

33. A method for analyzing data obtained by a determination method according to any one of claims 1, 12, 13, 19-22 and 27-32, further comprising correcting an intensity value of fluorescence of a reaction system, said intensity value being available after said target nucleic acid has hybridized to said nucleic acid probe labeled with said fluorescent dye, in accordance with an intensity value of fluorescence of said reaction system available after a probe-nucleic acid hybrid complex so formed has dissociated.

34. A method for analyzing data obtained by a real-time quantitative PCR method according to claim 32, further comprising, as a correction processing step, correcting an intensity value of fluorescence of a reaction system, said intensity being available in each cycle after said amplified nucleic acid has conjugated to said fluorescent dye or after said amplified nucleic acid has hybridized to said nucleic acid probe labeled with said fluorescent dye, in accordance with an



intensity value of fluorescence of said reaction system available after a nucleic acid-fluorescent dye conjugate or probe-nucleic acid hybrid complex so formed has dissociated in said cycle.

- 5           35. A method according to claim 34, wherein said correction-processing step is performed in accordance with the following formula (1) or formula (2):

$$f_n = f_{hyb,n} / f_{den,n} \quad (1)$$

$$f_n = f_{den,n} / f_{hyb,n} \quad (2)$$

10       where

$f_n$ : correction-processed value in an  $n^{th}$  cycle as calculated in accordance with the formula (1) or formula (2),

15        $f_{hyb,n}$ : intensity value of fluorescence of the reaction system available after said amplified nucleic acid has conjugated to said fluorescent dye or said amplified nucleic acid has hybridized to said nucleic acid probe labeled with said fluorescent dye in said  $n^{th}$  cycle, and

20        $f_{den,n}$ : intensity value of fluorescence of the reaction system available after said nucleic acid-fluorescent dye conjugate has dissociated in said  $n^{th}$  cycle or said probe-nucleic acid hybrid complex has dissociated in said  $n^{th}$  cycle.

- 25           36. A method according to claim 35, which comprises:

introducing correction-processed values, which have been calculated in accordance with the formula (1) or formula (2) in individual cycles, into the following formula (3) or (4) to calculate rates or percentages of changes in fluorescence between samples in said individual cycles:

$$F_n = f_n / f_a \quad (3)$$

$$F_n = f_a / f_n \quad (4)$$

where

$F_n$ : rate or percentage of a change in fluorescence in an  $n^{\text{th}}$  cycle as calculated in accordance with the formula (3) or formula (4),

$f_n$ : correction-processed value calculated in said  $n^{\text{th}}$  cycle as calculated in accordance with the formula (1) or formula (2), and

$f_a$ : correction-processed value calculated in a given cycle before a change in  $f_n$  is observed as calculated in accordance with the formula (1) or formula (2); and comparing said rates or percentages of changes in fluorescence.

37. A method according to claim 36, which comprises the following processing steps:

1) performing processing in accordance with the following formula (5), (6) or (7) by using data of rates or percentages of changes in fluorescence as calculated in accordance with said formula (3) or (4):

$$\log_b(F_n), \ln(F_n) \quad (5)$$

$$\log_b\{(1-F_n) \times A\}, \ln\{(1-F_n) \times A\} \quad (6)$$

$$\log_b\{(F_n-1) \times A\}, \ln\{(F_n-1) \times A\} \quad (7)$$

where

5        A,b: desired numerical values, and

$F_n$ : rate or percentage of a change in fluorescence in an  $n^{\text{th}}$  cycle as calculated in accordance with the formula (3) or formula (4),

10        2) determining a cycle in which said processed value of said processing step 1) has reached a constant value,

3) calculating a relational expression between cycle of a nucleic acid sample of a known concentration and the number of copies of said target nucleic acid at the time of initiation of a reaction, and

15        4) determining the number of copies of said target nucleic acid in an unknown sample upon initiation of PCR.

38. A method for analyzing a melting curve of a target nucleic acid, which comprises:

20        performing PCR on said target nucleic acid by using a nucleic acid probe according to any one of claims 2-11; and analyzing said melting curve of said target nucleic acid to determine a  $T_m$  value of each amplified nucleic acid.

39. A method for analyzing a melting curve of a target nucleic acid, which comprises:

25        performing PCR on said target nucleic acid by using a

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nucleic acid probe according to claim 4 or 7 as a primer; and  
analyzing said melting curve of said target nucleic acid  
to determine a  $T_m$  value of each amplified nucleic acid.

40. A data analyzing method for a real-time quantitative  
5 PCR method according to claims 34-37, which comprises the  
following steps:

gradually heating a PCR-amplified nucleic acid from a low  
temperature until complete denaturation of said nucleic acid;  
measuring an intensity of fluorescence at predetermined  
10 time intervals during said heating step;

displaying results of said measurement as a function of  
time on a display such that a melting curve of said nucleic acid  
is drawn on said display;

differentiating said melting curve to obtain  
15 differentiated values ( $-dF/dT$ ,  $F$ : intensity of fluorescence,  
 $T$ : time);

displaying said differentiated values as derivatives on  
said display; and

determining a point of inflection from said derivatives.

41. A determining and/or analyzing system for real-time  
20 quantitative PCR, comprising:

means for performing a data analysis step by a data  
analysis method according to claim 34;

means for performing a data analysis step by a data  
25 analysis method according to claim 35;

means for performing a data analysis step by a data analysis method according to claim 36;

means for performing a data analysis step by a data analysis method according to claim 37; and

5 means for performing a data analysis step by a data analysis method according to claim 40.

42. A computer-readable recording medium comprising a program recorded therein for making a computer perform the following steps:

10 analysis of data by a data analysis method according to claim 34;

analysis of data by a data analysis method according to claim 35;

15 analysis of data by a data analysis method according to claim 36;

analysis of data by a data analysis method according to claim 37; and

analysis of data by a data analysis method according to claim 40.

20 43. A method for quantitating a nucleic acid, which comprises using a data analysis method according to any one of claims 34-37 and 40 for real-time quantitative PCR.

44. A method for quantitating a nucleic acid, which comprises using a system according to claim 41.

25 45. A method for quantitating a nucleic acid, which

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